Photovoltaic $LiNbO_3$ particles: Applications to Biomedicine/Biophotonics

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Abstract

Recently, a novel method to trap and pattern ensembles of nanoparticles has been proposed and tested. It relies on the photovoltaic (PV) properties of certain ferroelectric crystals such as $LiNbO_3$ [1,2]. These crystals, when suitably doped, develop very high electric fields in response to illumination with light of suitable wavelength. The PV effect lies in the asymmetrical excitation of electrons giving rise to PV currents and associated space-charge fields (*photorefractive effect*). The field generated in the bulk of the sample propagates to the surrounding medium as evanescent fields. When dielectric or metal nanoparticles are deposited on the surface of the sample the evanescent fields give rise to either electrophoretic or dielectrophoretic forces, depending on the charge state of the particles, that induce the trapping and patterning effects [3,4].

The purpose of this work has been to explore the effects of such PV fields in the biology and biomedical areas. A first work was able to show the necrotic effects induced by such fields on He-La tumour cells grown on the surface of an illuminated iron-doped $LiNbO_3$ crystal [5]. In principle, it is conceived that $LiNbO_3$ nanoparticles may be advantageously used for such biomedical purposes considering the possibility of such nanoparticles being incorporated into the cells. Previous experiments using microparticles have been performed [5] with similar results to those achieved with the substrate. Therefore, the purpose of this work has been to fabricate and characterize the $LiNbO_3$ nanoparticles and assess their necrotic effects when they are incorporated on a culture of tumour cells.

Two different preparation methods have been used: 1) mechanical grinding from crystals, and 2) bottom-up sol-gel chemical synthesis from metal-ethoxide precursors. This later method leads to a more uniform size distribution of smaller particles (down to around 50 nm). Fig. 1(a) and 1(b) shows SEM images of the nanoparticles obtained with both method.

An *ad hoc* software taking into account the physical properties of the crystal, particullarly donor and aceptor concentrations has been developped in order to estimate the electric field generated in noparticles. In a first stage simulations of the electric current of nanoparticles, in a conductive media, due to the PV effect have been carried out by MonteCarlo simulations using the Kutharev 1-centre transport model equations [6] . Special attention has been paid to the dependence on particle size and $[Fe^{2+}]/[Fe^{3+}]$. First results on cubic particles shows large dispersion for small sizes due to the random number of donors and its effective concentration (Fig 2).

The necrotic (toxicity) effect of nanoparticles incorporated into a tumour cell culture subjected to 30 min. illumination with a blue LED is shown in Fig.3. For each type of nanoparticle the percent of cell survival in dark and illumination conditions has been plot as a function of the particle dilution factor. Fig. 1a corresponds to mechanical grinding particles whereas 1b and 1c refer to chemically synthesized particles with two oxidation states. The light effect is larger with mechanical grinding nanoparticles, but dark toxicity is also higher. For chemically synthesized nanoparticles dark toxicity is low but only in oxidized samples, where the PV effect is known to be larger, the light effect is appreciable.

These preliminary results demonstrate that Fe:LiNbO· nanoparticles have a biological damaging effect on cells, although there are many points that should be clarified and much space for PV nanoparticles optimization. In particular, it appears necessary to determine the fraction of nanoparticles that become incorporated into the cells and the possible existence of threshold size effects.

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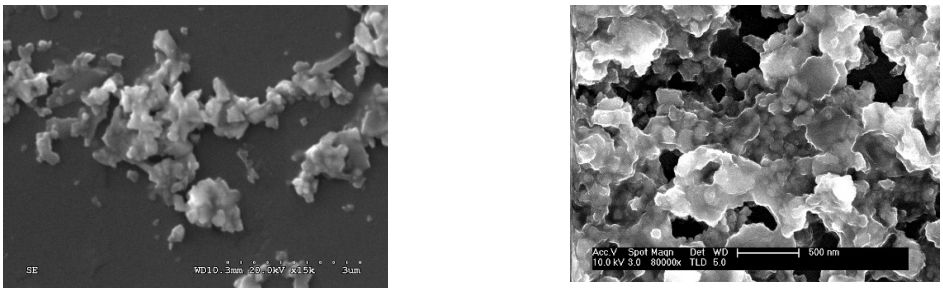
Figures



(a) (b)

Figure 1.- SEM imagines of $LiNbO_3$:Fe nanoparticles obtained by: (a) mechanical grinding, (b) chemical synthesis.

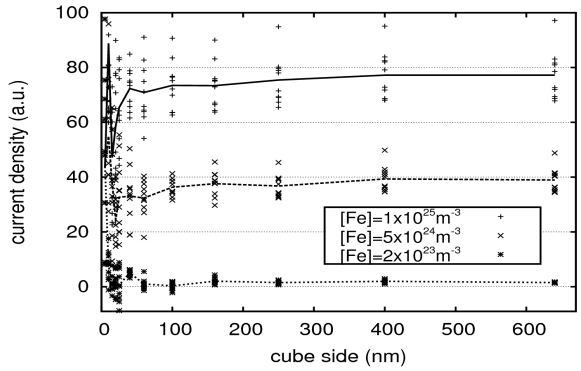
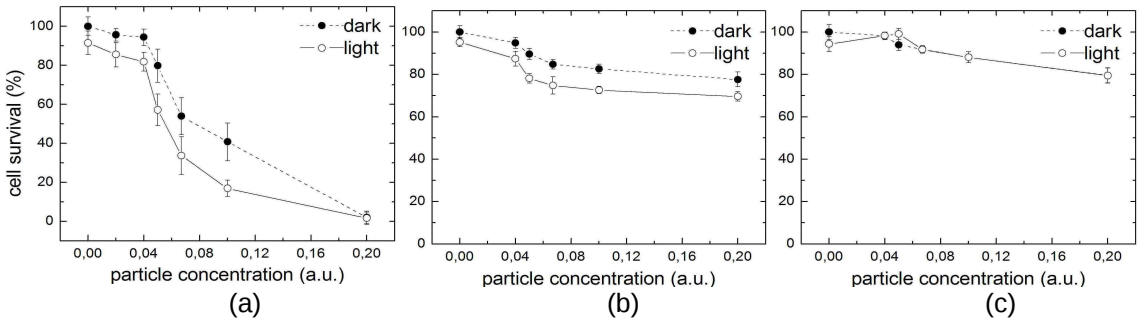


Figure 2.- Current density as a function of particle size. Dots represent individual particles. Lines connect mean current density for each size.



(a) (b) (c)

Figure 3.- Percent cell survival versus nano-particles concentration in solution obtained by mechanical grinding (a) and obtained by chemical synthesis and oxidized (b) and reduced (c) by thermal treatments.

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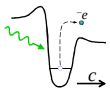
Introduction

❑ Bulk Photovoltaic Effect (BPE) in $LiNbO_3$

➢ ***Asymmetrical*** *optical transitions from impurities (Fe^{2+}, Cu^{+}) to the conduction band.*

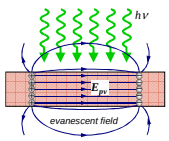


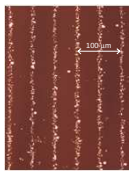
➢ ***Low*** *electrical current (photovoltaic current j_{pv}) that induces charge-carrier separation and a bulk* ***high electric field*** *(photovoltaic field, E_{pv}).*

$$E_{pv} = \frac{j_{pv}}{\sigma} = \frac{l_{pv}\gamma N_A}{\mu}$$

E_{pv} ~10^4 – 10^5 V/cm
(for visible light in $LiNbO_3$:Fe)

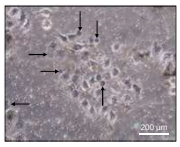
Evanescent electric fields are high enough to trap and pattern micro- nanoparticles on the surface crystal [1,2].





Trapping and patterning of 100 nm Ag particles induced by sinusoidal illumination in a $LiNbO_3$:Fe crystal.

These fields also have been able to induce necrotic death in tumour cells grown on the surface of a $LiNbO_3$:Fe crystal or cultured with microparticles of the same material [3].



Necrotic HeLa cells (pointed by arrows) in a culture with LiNbO3:Fe microparticles induced after 1 h visible illumination (optical microscopy image)..

❑ Main objective

➢ *The purpose of this work has been to explore the influence of the photovoltaic field in biomedical applications.*

➢ *$LiNbO_3$:Fe nanoparticles have been fabricated and characterized and their necrotic effects have been assessed after their incorporation in a culture of tumour (HeLa) cells.*

$LiNbO_3$ nanoparticles

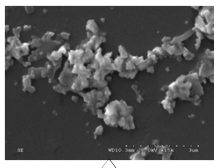
❑ **Mechanical Grinding (MG):** from congruent $LiNbO_3$ with 0.1 wt% Fe doping crystalline samples.

- grinding dissolved in PBS* (100 gr/l), decanted after 24 h sedimentation
- reduction/oxidation ratio $[Fe^{2+}]/[Fe^{3+}]$ ~ 0.05
- nanoparticle size ~ 200 – 400 nm

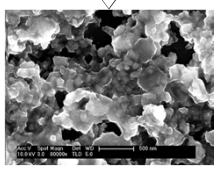
*Phosfate buffered saline

❑ **Chemical Synthesis (CS):** bottom-up sol-gel from metal (Li, Nb and Fe)-ethoxide precursors.

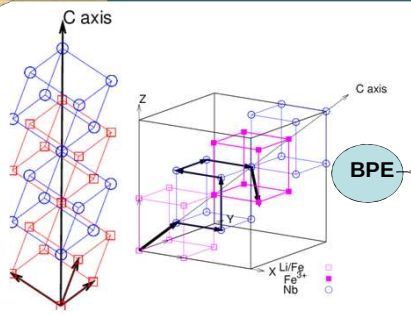
- Fe-doping 1 mol%
- crystalline phase verified by XRD
- thermal reduction in vacuum at 500 ºC
- mean crystalline size ~ 50 nm (obtained by XRD)
- nanoparticles also dissolved in PBS* (100 gr/l) and decanted after 24 h sedimentation



SEM images



BPE simulation in nanoparticles

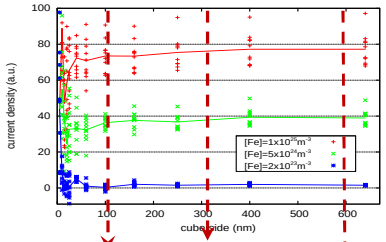


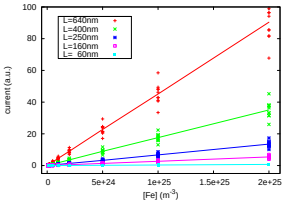
Simulated by MonteCarlo numerical experiments

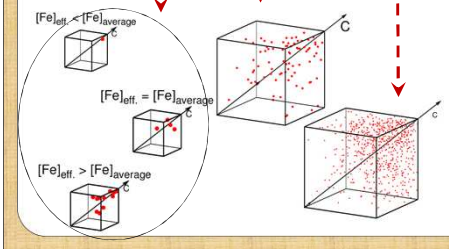
BPE

Due to
- e^- excitation from a Fe^{2+}
- random walk
- recombination in a Fe^{3+}

Assumptions
- Li/Fe sub-lattice supossed cubic
- Only excitation are considered
- Crystals are in a conductive medi







✓ For a given weight of LiNbO3 the smaller the crystal size the bigger the current density.

✓ For very small grain size (<60um), a big increase in the BPE may be found if individuals with greatest amount of Fe atoms can be isolated.

Nanoparticles effects in tumour HeLa cells

❑ Photo-Bioassay protocol

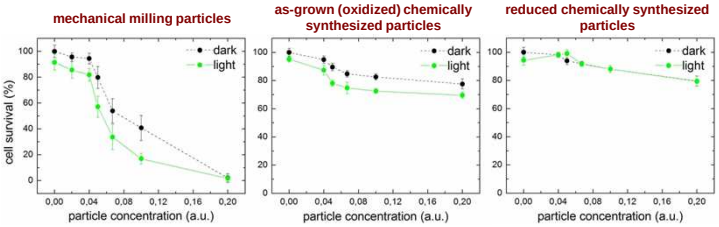
Cell cultures were incubated (for 3 – 24 h) with different solutions of nanoparticles, washed and exposed to visible light (30 min., LED of 1W 470-480 nm emission).
Identical cell cultures were not illuminated (dark conditions) for the purpose of controlling assays.

❑ Evaluation method

Cell mortality (in dark and lighting conditions) was evaluated by MTT cell viability assay technique.

❑ Results

The percentage of cell survival in dark and illumination conditions as a function of the particle dilution factor has been plot for each type of nanoparticle.



➢ *The effect of light on cell death is appreciable in cultures incubated with MG nanoparticles, small in cultures with oxidized CS and null in cultures with reduced CS nanoparticles.*

➢ *MG nanoparticles have a high dark toxicity, about 60% for medium particle concentrations, where the light effect is larger. However, for CS nanoparticles dark toxicity is low (20%).*

❑ Questions

✓ What fraction of nanoparticles has been incorporated into the cells?
✓ Is there a threshold effect in reducing nanoparticle size?

Summary

❑ Fe:$LiNbO_3$ nanoparticles have been fabricated by two different methods and their effect on HeLa cell cultures has been evaluated.

❑ The results demonstrate that nanoparticles have a biological damaging effect on tumour cells.

❑ The effect of light on cell death is in agreement with a high BPE for large particles and high Fe^{3+} concentration.

❑ The rule of BPE is not clear yet because of dark toxicity, and more work is necessary.

Acknowledgements: This work has been supported by MINECO under grant MAT2011-28379-C03.

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